Chapter 2

Relation of Coronary Microvascular Dysfunction in Hypertrophic Cardiomyopathy to Contractile Dysfunction Independent from Myocardial Injury


Abstract

**Background:** We studied the spatial relationships between hyperemic myocardial blood flow (hMBF), contractile function, and morphological tissue alterations in 19 patients with hypertrophic cardiomyopathy (HC).

**Methods:** All patients were studied with \[^{15}O\]water PET during rest and adenosine administration to assess myocardial perfusion. CMR was performed to derive delayed contrast enhancement (DCE) images and to calculate contractile function (E\(_{cc}\)) with tissue tagging. Eleven healthy subjects underwent similar PET and CMR scanning protocols and served as a control group.

**Results:** In the HC group, hMBF averaged 2.46 ± 0.91 mL/min/g and mean E\(_{cc}\) was -14.7±3.4%, both being decreased compared to the control group (3.97±1.48 mL/min/g and -17.7 ± 3.2% respectively, both P < 0.001). DCE was only present in HC patients, averaging 6.2±10.3% of LV mass. In the HC group, E\(_{cc}\) and DCE in the septum (-13.7±3.6% and 10.2±13.6%) significantly differed from the lateral wall (-16.0±2.8% and 2.4±5.9%, both P < 0.001). In general, hMBF and E\(_{cc}\) were reduced in segments displaying DCE, compared to nonenhanced segments (both P < 0.001). In the HC group, univariate analysis revealed relations of hMBF with E\(_{cc}\) (r = -0.45, P < 0.001) and DCE (r = -0.31, P < 0.001). Multivariate analysis revealed that E\(_{cc}\) was independently related to hMBF (β = -0.37, P < 0.001) and DCE (β = 0.28, P < 0.001).

**Conclusion:** In HC, hyperemic MBF is impaired and related to contractile function, independent from the presence of DCE. When present, DCE reflected a progressed disease state as characterized by an increased perfusion deficit and contractile dysfunction.
**Introduction**

Microvascular dysfunction is a common feature in patients with hypertrophic cardiomyopathy (HC), despite an angiographically normal coronary artery anatomy. (1) Consequently, hyperemic myocardial blood flow (hMBF) is hampered. (2-8) It has been postulated that microvascular dysfunction predisposes to myocardial ischemia, resulting in contractile dysfunction and scar formation. (9) Indeed, hMBF measured with first pass cardiovascular magnetic resonance imaging (CMR) or $^{13}$N-ammonia positron emission tomography (PET) has been related to wall thickening and delayed contrast enhanced (DCE) CMR. (5,7) However, scar tissue affects the kinetics of gadolinium and uptake of $^{13}$N-ammonia (10,11) and thus, estimates of perfusion. In addition, systolic wall thickening has a limited accuracy as a marker of contractile function in the presence of left ventricular hypertrophy (LVH). $^{15}$O-labelled water PET ($[^{15}\text{O}]_{\text{water}}$), however, determines perfusion in perfusable tissue only, excluding scar tissue from its MBF estimates. In addition, CMR tissue tagging yields data on myocardial systolic deformation, independent from wall thickness, thereby serving as a more reliable index of contraction in. (12) Therefore, the present study was conducted to investigate the interrelationships between hMBF, regional contractile function, and myocardial injury using $[^{15}\text{O}]_{\text{water}}$ PET and CMR tissue tagging and DCE.

**Methods**

Nineteen patients with symptomatic HC were enrolled in the study. HC was diagnosed according to the presence of LV septal hypertrophy on two-dimensional echocardiography (maximal wall thickness >15 mm in adults or >13 mm in relatives of a HC patient), in the absence of any other systemic or cardiac disease. (13) All patients exhibited asymmetrical LVH, located at the interventricular septum. None of the patients had significant ST-segment or T-wave abnormalities during resting electrocardiography, and all underwent coronary angiography to exclude the presence of coronary artery disease. The use of medication at the time of enrolment was not discontinued during the study. Eleven healthy volunteers, with normal electrocardiogram, normal physical examination, and without a relevant medical history were included as a control group. The study protocol was approved by the Medical Ethics Review Committee of the VU University Medical Center, Amsterdam, The Netherlands.

All PET scans were acquired by use of an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA) in two-dimensional mode. All patients were monitored with single-lead electrocardiography and blood pressure and heart
rate were recorded at regular intervals during the PET studies. A transmission
scan was performed using three rotating rod sources filled with $^{68}$Ga/$^{68}$Ge
solution. Subsequently, 1100 MBq $^{15}$O-water, dissolved in a 5 mL saline solution,
was injected intravenously, followed by a 40 mL saline solution flush at a rate of
4 mL/s. A dynamic emission scan was acquired, consisting of 40 frames with
variable frame length for a total time of 10 min (12*5, 12*10, 6*20, and 10*30 s).
After the rest study, myocardial blood flow (MBF) was determined during
hyperemia by infusion of adenosine at a rate of 140 µg per kilogram of body
weight per minute. Emission data were corrected for physical decay of $^{15}$O, dead
time, scatter, randoms and photon attenuation.

CMR studies were performed on a 1.5-Tesla whole body scanner (Magnetom
Sonata, Siemens, Erlangen, Germany), using a six-channel phased-array body coil.
After survey scans, a retro-triggered, balanced steady-state free precession
gradient-echo sequence was used for cine imaging. Image parameters were: slice
thickness 5 mm, slice gap 5 mm, temporal resolution <50 ms, repetition time 3.2
ms, echo time 1.54 ms, flip angle 60 degrees and a typical image resolution of
1.3*1.6 mm. The number of phases within the cardiac cycle was set at twenty.
Four-, 3-, and 2-chamber view cines were obtained.(14) Then, a stack of 10-12
short axis slices covering the LV was used for assessing LV volumes, mass and
ejection fraction (EF). The method of planning the image acquisition for LV
coverage has been described previously.(15) Cine images were acquired during
one breath-hold in mild expiration. Cine imaging with myocardial tagging was
applied to create noninvasive markers (tags) within the myocardium for the
calculation of strain.(16) Five short-axis tagged images with complementary
spatial modulation of magnetization tagging for improved strain calculations
were acquired as previously described.(17)

Delayed contrast enhanced (DCE) images were acquired 10-15 minutes after
intravenous administration of 0.2 mmol/kg gadolinium, by using a two-
dimensional segmented inversion-recovery prepared gradient-echo sequence.
Inversion-recovery time was 250-300 ms.

Data were transferred to a SUN workstation (SUN Microsystems Inc.) and
analyzed using Siemens/CTI software, together with additional tracer kinetic
analysis software developed in MATLAB. Reconstruction of the $^{15}$O-water
emission sinograms was performed using filtered back projection with a
Hanning filter at 0.5 of the Nyquist frequency, resulting in a transaxial spatial
resolution of $\approx$ 7 mm full width at half-maximum. Regions of interest (ROIs) were
deefined manually on $^{15}$O-water short axis summed uptake images at basal,
midventricular and apical levels of the LV according to a 12-segment model as
described previously in detail.(10) This set of ROIs was projected onto the
dynamic $^{15}$O-water images to generate time-activity curves (TACs). Additional ROIs were defined in the left atrial and right ventricular bloodpool. This latter set of ROIs was projected onto the dynamic $^{15}$O-water images to generate TACs of intravascular activity. These latter TACs were used as image-derived input functions and for use in spill-over correction of myocardial tissue TACs. Using a standard single tissue compartment model, MBF (mL/min/g of perfusable tissue) was determined for all myocardial $[^{15}$O]water TACs.(18) Average MBF was calculated by grouping all ROIs, whereas the regional blood flows were calculated by grouping specific ROIs. Corrections were made for left and right ventricular spillover effects by use of the method described by Hermansen et al.(19) In addition, resting coronary microvascular resistance (CMVR) was calculated by dividing mean arterial pressure (MAP) with MBF, whilst minimal CMVR was derived in a similar fashion, only during hyperemic conditions.(20)

Left ventricular volume analysis was performed by manually drawing epicardial and endocardial contours on all end-diastolic (ED) and end-systolic (ES) LV short-axis images. Global LV function parameters, including ED volume (LVEDV), ES volume (LVESV), ejection fraction (LVEF), and myocardial mass, were then derived from the cine images with use of the MASS software package (MEDIS, Leiden, The Netherlands). The tagging images were used to generate circumferential strain curves for each myocardial segment. Subsequently, $E_{cc}$, which indicates maximum contractile function, was derived for each segment from the strain curves.(17) Similar segmentation for average and regional analyses of $E_{cc}$ was used as described for the PET data. Since circumferential shortening is determined by the shortening of myofibers, $E_{cc}$ is expressed as a negative value.

Finally, each myocardial segment was evaluated for the presence of hyperenhancement, which was defined as an area of signal enhancement greater than 5 SD of the signal of nonenhanced myocardium. The extent of DCE was expressed as the percentage of the total myocardial tissue area studied. Similar segmentation for average and regional analyses of myocardial hyperenhancement was used as described for the PET data.

Results are displayed as mean ± SD. For the comparison of two data sets, unpaired Students t-test was used. For the comparison of multiple data sets, one-way analysis of variance (ANOVA) was applied with post hoc Bonferroni adjustment for inequality. Correlations between variables were evaluated with univariate and multivariate analysis. All tests were performed two-sided and a $P$ value < 0.05 was considered statistically significant.
Results

Baseline characteristics of the HC and control study population are presented in table 1. All HC patients except four, in whom side effects were considered intolerable, used β-receptor blockers and/or Ca²⁺ channel-blockers, the subtypes and dosages of which are specified in table 2. Hemodynamic parameters obtained during the rest and hyperemic PET studies are shown in table 3.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HC</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 19)</td>
<td></td>
<td>(n = 11)</td>
<td></td>
</tr>
<tr>
<td>Men/Women</td>
<td>11/8</td>
<td>7/4</td>
<td>0.77</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>55 ± 14</td>
<td>53 ± 3</td>
<td>0.86</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>2.1 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>0.72</td>
</tr>
<tr>
<td>Left ventricular mass (g)</td>
<td>178 ± 58</td>
<td>99 ± 21</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Left ventricular end-diastolic volume (mL)</td>
<td>191 ± 33</td>
<td>179 ± 33</td>
<td>0.80</td>
</tr>
<tr>
<td>Left ventricular end-systolic volume (mL)</td>
<td>76 ± 20</td>
<td>73 ± 21</td>
<td>0.72</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>59 ± 8</td>
<td>61 ± 5</td>
<td>0.98</td>
</tr>
<tr>
<td>LV outflow tract obstruction (&gt;30 mmHg)</td>
<td>5 (26%)</td>
<td>0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NH₂-terminal pro-brain natriuretic peptide (ng·L⁻¹)</td>
<td>535 ± 536</td>
<td>61 ± 53</td>
<td>&lt; 0.001</td>
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</tbody>
</table>

Table 1. Baseline characteristics. HC, hypertrophic cardiomyopathy.
Table 2. Medication specifications of hypertrophic cardiomyopathy patients

<table>
<thead>
<tr>
<th>Medication</th>
<th>Patients (n)</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Metoprolol 100 mg</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Metoprolol 50 mg</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Bisoprolol 2.5 mg</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Metoprolol 50 mg, Diltiazem 300 mg</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Bisoprolol 2.5 mg, Verapamil 180 mg</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Metoprolol 100 mg, Verapamil 80 mg</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Verapamil 240 mg</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Diltiazem 200 mg</td>
<td>1 (5%)</td>
</tr>
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</table>

Table 3. Hemodynamic parameters during positron emission tomography

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Hyperemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>Controls</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130 ± 25</td>
<td>124 ± 16</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75 ± 9</td>
<td>73 ± 8</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>94 ± 13</td>
<td>90 ± 10</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>61 ± 9</td>
<td>66 ± 11</td>
</tr>
</tbody>
</table>
Table 4 represents the regional resting and hyperemic MBF, E\textsubscript{cc}, and DCE for both study groups. A total of 228 segments were analyzed in the HC group and 132 in the control group (12 segments per subject). In the HC group, resting MBF averaged 0.96 ± 0.21 mL/min/g and was slightly lower compared to the control group (1.09 ± 0.36 mL/min/g, \( P = 0.10 \)). MBF was significantly decreased in the hypertrophied septum and the inferior wall, compared to the lateral wall (\( P < 0.01 \) and \( P < 0.05 \), respectively). Hyperemic MBF in the HC group averaged 2.46 ± 0.91 mL/min/g, which was significantly lower compared to the control group (3.97 ± 1.48 mL/min/g, \( P < 0.001 \)). In both study groups, hMBF was distributed homogeneously across all regions. E\textsubscript{cc} in the HC group averaged -14.7 ± 3.4\%, which was significantly lower compared to controls (-17.7 ± 3.2\%, \( P < 0.001 \)). In addition, E\textsubscript{cc} was distributed more heterogeneously in HC, with significant impairment of E\textsubscript{cc} in the hypertrophied septum compared to the lateral wall (\( P < 0.001 \)). On average, 6 ± 10\% of the myocardium displayed DCE in HC, predominantly localized in the basal anteroseptal region at the junction with the right ventricular free wall (14 ± 12\%). Mean ED wall thickness of the hypertrophied septum was 16.3 ± 2.8 mm. Analysis of all myocardial HC segments revealed that hMBF decreased in proportion to the increase in ED wall thickness (Figure 1, \( P < 0.001 \), ANOVA). To the contrary, the extent of DCE increased in proportion to the increase in ED wall thickness (\( P < 0.001 \), ANOVA).

![Figure 1](image_url)

**Figure 1.** Bar charts demonstrating that hMBF (A) and E\textsubscript{cc} (B) are significantly impaired in regions that display DCE (black bars) compared with nonenhanced segments (white bars).
Table 4. Regional positron emission tomography and cardiovascular magnetic resonance data for hypertrophic cardiomyopathy patients (n = 19) and controls (n = 11). In the HC group, the P values (as determined by ANOVA) for MBF, hMBF, E\textsubscript{cc} and DCE were: P = 0.002, P = 0.70, P < 0.001 and P < 0.001, respectively. In the control group, the P values (as determined by ANOVA) for MBF, hMBF, E\textsubscript{cc} and DCE were: P = 0.08, P = 0.15, P = 0.05 and P = 1.

*P < 0.05 versus septal
†P < 0.01 versus inferior
‡P < 0.001 versus lateral

<table>
<thead>
<tr>
<th>Region</th>
<th>MBF (mL/min/g)</th>
<th>Hyperemic MBF (mL/min/g)</th>
<th>E\textsubscript{cc} (%)</th>
<th>DCE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>Controls</td>
<td>HC</td>
<td>Controls</td>
</tr>
<tr>
<td>Anterior</td>
<td>1.00 ± 0.22</td>
<td>1.18 ± 0.32</td>
<td>2.36 ± 1.01</td>
<td>4.40 ± 1.37</td>
</tr>
<tr>
<td>Lateral</td>
<td>1.02 ± 0.27*†</td>
<td>1.18 ± 0.35</td>
<td>2.54 ± 0.99</td>
<td>3.73 ± 1.30</td>
</tr>
<tr>
<td>Inferior</td>
<td>0.86 ± 0.24</td>
<td>0.96 ± 0.30</td>
<td>2.43 ± 1.23</td>
<td>4.37 ± 2.02</td>
</tr>
<tr>
<td>Septal</td>
<td>0.90 ± 0.24</td>
<td>1.05 ± 0.40</td>
<td>2.34 ± 1.02</td>
<td>3.80 ± 1.35</td>
</tr>
<tr>
<td>Average</td>
<td>0.96 ± 0.21</td>
<td>1.09 ± 0.36</td>
<td>2.46 ± 0.91</td>
<td>3.97 ± 1.48</td>
</tr>
</tbody>
</table>
During resting conditions, the CMVR was 102 ± 22 mmHg · mL · min⁻¹ in the HC group, which was not significantly different from the control group (86 ± 19 mmHg · mL · min⁻¹, P = 0.07). CMVR during hyperemic conditions, however, was significantly higher compared to the controls (37 ± 15 mmHg · mL · min⁻¹ versus 22 ± 6 mmHg · mL · min⁻¹, P = 0.004).

Figure 2A and 2B demonstrate that hMBF and Eₜ were significantly reduced in segments displaying DCE (n = 120), compared to the segments without DCE (n = 108) (both P < 0.001) in the HC group. In myocardial HC segments without DCE, hMBF was significantly affected by the extent of contractile dysfunction (expressed as Eₜ, ranging from normal to severely impaired) (Figure 3A, P < 0.001, ANOVA). A similar relationship between hMBF and Eₜ was found when only the segments with DCE were analyzed (Figure 3B, P < 0.001, ANOVA). Nonenhanced segments exhibiting severely impaired Eₜ (n = 5) were exceptional, as well as hyperenhanced segments with normal Eₜ (n = 8), with an average DCE extent of only 1 ± 2%. In the HC group, univariate analysis demonstrated that regional hMBF was inversely related to Eₜ (r = -0.45, P < 0.001) as well as to the extent of DCE (r = -0.31, P < 0.001). A linear relationship was also observed between the extent of DCE and Eₜ (r = 0.39, P < 0.001). Multivariate analysis revealed that Eₜ was independently related to hMBF (β = -0.37, P < 0.001) and DCE (β = 0.28, P < 0.001). Figure 4 demonstrates these relationships in scatter plots.

![Figure 2](image-url)

**Figure 2.** Bar charts depicting hMBF values for all HC segments (A) without DCE (white bars) and varying degrees of contractile function, categorized as normal (>18.0%), moderately (18.0%-11.5%), and severely (<11.5%) impaired. B. Idem, but for HC segments with DCE (black bars).
Figure 3. Bar charts depicting hMBF values (hatched bars) and extent of DCE (black bars) in relation to end-diastolic wall thickness. ANOVA was significant for both trends ($P < 0.001$).
Discussion

In HC patients, coronary microvascular dysfunction results in blunted hyperemic myocardial perfusion (hMBF), especially in areas with pronounced hypertrophy, typically the interventricular septum. (5,6) Similarly, patients with LVH due to hypertension or aortic stenosis also have reduced hMBF, (21,22) implying that pathological hypertrophy itself substantially impedes myocardial perfusion, presumably due to a relative decrease in capillary density. Interestingly, hMBF was homogeneously reduced across the entire myocardium. Hence, microvascular dysfunction is not solely confined to the septum, but appears as a widespread phenomenon, also affecting non-hypertrophied areas. (2,8) These observations suggest diffuse impairment of microvascular function, which is
supported by histological findings of intramural coronary artery remodeling throughout the LV myocardium. In addition, increased coronary microvascular resistance as a result of augmented extravascular compressive forces, due to left ventricular outflow tract obstruction, have independently been related to microvascular dysfunction as well. 

Resting MBF was significantly reduced in the hypertrophied septal regions compared to the lateral free wall, and has been linked with the interventricular localization of DCE. Correspondingly, the extent of DCE in the current HC study group was significantly largest in the septum, principally affecting the anteroseptal segment. The CMR tagging data revealed significant impairment of contractile function of the hypertrophied septum. Interestingly, contractile function of the non-hypertrophied lateral wall was also decreased, albeit to a much lesser degree than the septum. The main finding of this study was that regional hMBF in HC was significantly correlated to contractile function, irrespective of the presence of DCE. Furthermore, the extent of DCE was independently and inversely related to contractile function.

A blunted perfusion reserve in HC has been associated with loss of contractile function in other cross-sectional reports. In this study, the increase of MBF after adenosine infusion, i.e. the coronary vasodilatory reserve, was significantly blunted. Consequently, microvascular dysfunction in HC predisposes to myocardial ischemia, and may result in ischemic injury with loss of contractile function and secondary replacement fibrosis. Correspondingly, the current data revealed that hMBF decreased in proportion to the extent of contractile dysfunction, both in the absence and the presence of DCE. This hypothesis was recently corroborated by a follow-up study conducted by Olivotto et al, who demonstrated that HC patients with a severe perfusion reserve deficit are at increased risk for developing contractile dysfunction during follow-up, even in the absence of abnormal contractile function or tissue morphology at the start of the study. Histological examination of HC hearts has revealed a disorganized structure of cardiomyocytes in the hypertrophied septum, seemingly leading to a reduced contractile function on a macroscopic level with normal oxidative metabolism. Inasmuch as this myofiber disarray is presumably the result of mutations in the abnormal sarcomeric protein, it is at least in part considered to be an independent process upon microvascular capacity. Therefore, the presence of myocyte disarray alone could also contribute to the significant impairment of contractile function of the hypertrophied septum currently observed.

Hyperemic MBF was significantly more depressed in segments displaying DCE as compared to nonenhanced segments, which has been documented previously.
during hyperemic,(7) as well as resting myocardial perfusion PET studies.(25) Next to fibrosis, DCE is thought to reflect several other substrates in HC,(28) the interpretation of which is presumably dependent upon the stage of the disease process. In the acute or subacute ischemic phase, DCE may reflect inflammation, focal edema, and myocyte necrosis, whereas in the chronic phase, these hyperenhanced foci are likely to be the gross result of interstitial and replacement fibrosis.(29) The current data also demonstrated reduced hMBF values in the absence of DCE. This suggests that microvascular dysfunction constitutes a primary component of the HC phenotype, with secondary occurrence of ischemic injury and subsequent acute and chronic tissue responses, both reflected by DCE.(9) Concordant with the latter hypothesis are cross-sectional findings by Sotgia and coworkers who also observed correlations of impaired hMBF with DCE and contractile dysfunction.(7) It was shown that loss of perfusion reserve in DCE negative areas located nearby or adjacent to hyperenhanced areas, was more pronounced when compared to remote segments.(5,7) In our study, DCE also extended to the non-hypertrophied lateral wall, which could be explained by the fact that microvascular dysfunction is not solely confined to areas with hypertrophy, although it does correlate in severity with the magnitude of segmental hypertrophy.

In the current study population, nearly every myocardial segment with severe contractile dysfunction also displayed hyperenhancement. Extensive presence of DCE has been associated with significant impairment of contractile function in various other HC investigations.(26,29,30,31) Histological examination has confirmed that replacement and interstitial fibrosis are both distinctive features of end-stage HC hearts.(23) Accordingly, a contributable role for myocardial fibrosis in impeding systolic function, by mechanically interfering with myocardial shortening, should be considered.(17,25,26) This is underlined by the currently presented significant relationship between contractile dysfunction and hyperenhancement, regardless of hMBF.

Alltogether, these results imply that HC patients with relatively large perfusion defects are at increased risk of developing contractile dysfunction and myocardial fibrosis, with a concominant risk of heart failure and potentially lethal arrhythmias on the long term.(4) Hence, early detection of segments with abnormal perfusion may warrant the initiation of treatment strategies that could protect the myocardium against repetitive ischemic episodes, e.g. betablocker therapy,(32) or ameliorate perfusion defects in the presence of significant LVOT obstruction, by use of alcohol septal ablation/surgical myectomy.(6)

A limitation of the current study is the small sample size, which could have limited the statistical accuracy of our results. This is especially relevant when
aspects of the myocardium are compared on a regional and/or segmental level, where absolute differences in and between study groups are small. Furthermore, drug treatment was sustained because of ethical reasons.

Whereas Ca\(^{2+}\) channel-blocker agents exert little effect on resting and hyperemic blood flow,\(^{(33)}\) \(\beta\)-receptor blockers have been shown to significantly reduce stroke volume and EF, and therefore their effect on regional contractile function may be substantial.\(^{(34)}\) This might have influenced our results and has introduced bias compared with the control group.
Reference list

ventricular filling assessed using cardiac magnetic resonance imaging in healthy subjects. Am J Cardiol 2007;100:122-127.


